THE USE OF D-TUBOCURARINE CHLORIDE IN ANÆSTHESIA

Lecture delivered at The Royal College of Surgeons of England
on
17th April, 1947
by

T. Cecil Gray, M.D., D.A.
Reader in Anæsthesia, University of Liverpool

THE SUBJECT of curare has developed to such an extraordinary extent during the past two or three years that it is now quite impossible to review it in any detail in one lecture.

So much work has been done on its chemistry, botany, pharmacology and its clinical applications that it is possible only to give the briefest outline and to emphasise some of the points which appear to me to be important.

Introduction and Origin of Curare

" Curare " is a name given to various highly poisonous substances used by the South American Indian tribes to poison their arrows. These tribes dwell in the regions between the Amazon and Orinoco rivers. The substances vary considerably in composition. Their story is well known and one has only time to mention the bare facts of their introduction into the civilized world. Sir Walter Raleigh was the first to describe them when he wrote about his discovery of British Guiana in 1595. Later the arrow poisons were mentioned by various missionaries, notable among them being the Jesuit Father d'Acugna, who, in his publication of 1642, described how he found in the Amazonian jungles " an abundance of venomous herbs, with which some of these natives make so subtile a poison, that their arrows being rub'd with it, never draw the least blood without taking the life at the same time."

The actual composition of these poisons remained for centuries a closely guarded secret but eventually during the nineteenth century such eminent botanists and explorers as Humbold and Bonpland (1821), Schomburgk (1857) and Waterton (1812), by virtue of personal observations carried out on the spot, were able to throw much light on the problem, and more recently the investigations of Mr. R. C. Gill (1946), an American who lived among the Indians for a considerable time, and Mr. Harold King, an English chemist, have enormously increased our knowledge on this subject.

Curare was prepared by the distillation of the barks of many plants. Some of these supplied the paralysing principle, others were added to

give the substance a suitable consistency, and finally other animal products such as the fangs of poisonous snakes and reptiles, ants and spiders, were added in an ignorant hope that they would increase the efficacy of the potion.

From the work of King (1935), Wintersteiner and Dutcher (1943), it seems clear that raw curare depends for its paralysant activity on the presence in it of one or more plants of two types. The strychnos toxifera of Schomburgk and the strychnos castelnaea both yield a physiologically active extraction. In the other group are many plants of the natural order Menispermaceae, chief among which is the chondodendron tomentosum.

In 1895 Boehm attempted a classification of crude curare according to the containers in which it was exported by the Indians. These containers were of three main types—a hollowed bamboo cane which contained " tube curare," a calabash or gourd containing " calabash curare," and an earthenware jar or pot from which came " pot curare." Boehm analysed these curares and found that he was able to obtain different substances from each variety. The substances were amorphous quaternary alkaloids with a high paralysant activity and crystalline tertiary alkaloids with practically no paralysant activity but which, in some cases, were toxic in other ways.

Variety of Curare	Type of Alkaloid	Name (Boehm)	Composition	Activity
Tube curare	Amorphous quaternary	Tubocurarine	$C^{19}H^{21}NO^{4}$	++++
	Crystalline tertiary	Curine	$C^{18}H^{19}O^{3}N$	+
Pot curare	Amorphous quaternary	Protocurarine	$C^{19}H^{20}O^{2}N$	+++
	Crystalline tertiary	Protocurine	$C^{20}H^{23}O^{3}N$	+
	Crystalline tertiary	Protocuridine	$C^{19}H^{21}O^{3}N$	+
Calabash curare	Amorphous quaternary	Curarine	$C^{19}H^{26}N^{2}O$	++++

The Table shows the Boehm classification of curare and the substances he obtained from it. Particular attention should be paid to tube curare. In this he found the highly active amorphous substance tubocurarine, which is the base of the substances Intocostrin and d-tubocurarine chloride, which are becoming of such extensive clinical use to-day, but he also found in it the very toxic tertiary base curine. This has a depressant action on the heart very similar to that exerted by digitalis. It is thus no wonder that crude curare was often too toxic to use clinically.

In 1935, King, of London, prepared from tube curare a crystalline alkaloid d-tubocurarine chloride. His work, for the first time, provided us with a substance of definite crystalline structure and composition and with constant pharmacological properties. This substance, prepared by Messrs. Burroughs Wellcome & Co. in a 10 per cent. solution, goes under the proprietary name of " Tubarine," and it is this product which has been used chiefly by us in England as an aid to anæsthesia.

In the United States, Gill had made available a large quantity of crude curare made chiefly from the chondodendron tomentosum. Messrs. E. R. Squibb prepared from this a total extract which was purified, biologically standardised, and put on the market under the name of " Intocostrin." Intocostrin is an amber coloured solution standardised to contain 20 mg. of crude curare to the millilitre. It has been shown by the American Council of Pharmacy and Chemistry (1945) that by animal assay 1 mg. of Intocostrin is equivalent in paralysant activity to 0·15 mg. of d-tubocurarine chloride. In clinical use it might be more correct to say that 1 mg. of Intocostrin produces an effect similar to that produced by 0·2 mg. of Tubarine.

Pharmacology

Investigations of the pharmacological action of crude curare had always been confused by its varying composition, but now the availability of these purer products has made possible more accurate observations.

Taken orally these substances have no effect in human beings, although some animals and birds, notably pigeons, are affected when the poison is eaten. It has been shown that this immunity in humans and most animals is not due to any action of the gastric or digestive juices but more to a slow absorption and rapid excretion. Curare is excreted in the urine and traces of curare activity can be found in the urine of animals and humans who have been treated with this substance. It is said also that curare is destroyed by the liver. The evidence for this is at the moment rather inconclusive and judgment should be withheld pending further investigations carried out with the pure substances now available.

The classical experiments on frogs, which Claude Bernard (1857) performed in the years following 1840, showed that the paralysant activity of curare was due to an interference in the conduction of nerve impulses from the motor nerve to the muscle and that this interference occurred at the myoneural junction. In the curarised animal both the nerve and the muscle are still capable of responding to stimuli, but the break in conduction occurs at the junction of the two.

The work of Dale, Feldberg, Vogt (1936) and Brown (1936) leads us to believe that on stimulation of a nerve to a voluntary muscle acetylcholine is produced at the neuromuscular junction, and that curare produces its paralysant effect by preventing the action of this on the receptor substance of the muscle. While on the subject it is useful to consider the

action of physostigmine, which is said to be the natural and physiological antidote to curare. Normally acetylcholine is neutralised by an enzyme, cholinesterase, present in the tissues and blood. Physostigmine or prostigmine prevents this neutralisation, and so allows an abnormal and excessive barrage of acetylcholine to play on the receptor substance; this may succeed in overcoming its inhibition by curare.

That is how curare produces paralysis, from the point of view of the chemical theory of neuromuscular transmission, but this action can also be regarded in the light of the electrical theory of nerve impulse conduction. If the changes in electrical potential which occur at a point in the muscle on stimulation of the motor nerve be recorded, a typical curve is obtained (Fig. 1).

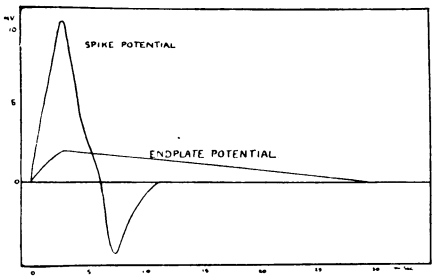

Fig. 1
Comparison between the spike potential of muscle and the end-plate potential recorded after complete curarisation—semi-diagrammatic. (From Fulton. Howell's Textbook of Physiology 15th ed.)

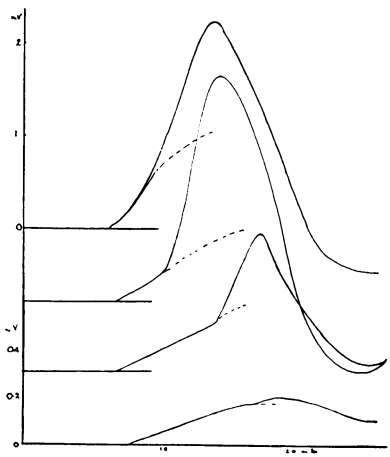

Fig. 2
Changes in the potential recorded at the end-plate region of a muscle as it becomes progressively curarised. Three things happen: 1. The spike potential decreases in amplitude; 2. It begins later in time; 3. The end-plate potential decreases in amplitude. Its supposed course is indicated by the hatched line extrapolating the first phase of the recorded potential. (From Fulton. Howell's Textbook of Physiology, 15th ed.)

This negative potential is called the "spike" potential owing to the shape of the curve. In the curarised muscle Eccles, Katz and Kuffler (1941) have been able to record the changes in the potential actually at the motor end-plate, that is at the myoneural junction, and a different type of curve is obtained. During progressive curarisation of a muscle it will be seen (Fig. 2) that the spike potential becomes progressively reduced, is later in onset, and that the end-plate potential is also reduced.

This produces a simple explanation of the curare action. By altering the time relationships of these potentials it may well be that the rate of rise in excitability in the motor end-plate, as indicated by the end-plate potential, is depressed by curare and the threshold necessary for stimulation of the muscle substance is never reached—the muscle is then completely curarised.

The truth is probably a combination of the chemical and electrical theories.

Injected intramuscularly the typical paralysant activity becomes apparent in about 20 minutes, but when given intravenously the action appears in 10 seconds and is fully developed in two minutes. The first muscles to be affected are the extrinsic muscles of the eyes. At this stage the subject may complain of tiredness, inability to keep his eyes open, and diplopia. The muscles of the face and tongue are next affected followed in turn by those of the neck, the deep vertebral muscles, those of the limbs and thorax, and finally the diaphragm with resultant complete respiratory paralysis. The animal or the patient at this stage, unless supported by artificial respiration, dies from asphyxia.

The point which must be stressed at this juncture is that although, in the average case, this sequence of events is roughly correct there are still variations in reaction. One subject, a volunteer, received 45 mg. of d-tubocurarine chloride, which is a very large dose, and although all respiratory activity was abolished he was still able to move his hands. There is, moreover, a marked overlap in the sequence of the paralysis.

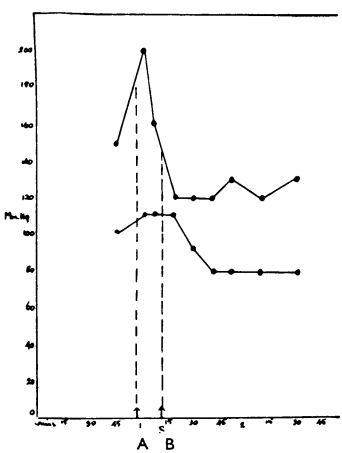

Fig. 3

Blood pressure recordings of a male patient aged 50 undergoing partial gastrectomy and anæsthetised with Thiopentone and d-tubocurarine chloride. To show the elevation of blood pressure due to under-ventilation.

It is very important to realise that any dose of curare which produces a clinical effect such as abdominal muscular relaxation also affects to some extent the intercostal muscles and the diaphragm, although they may not be completely paralysed and the patient may continue to breathe. The tidal air is thus always reduced and, although the patient may be kept well oxygenated by the administration of a high percentage of oxygen, the fact remains that his tidal exchange is insufficient, the ventilation of his lungs is thus impaired and inadequate, an increased tension of carbon dioxide in the blood occurs, the blood pressure rises and a troublesome oozing of blood in the site of operation may be seen. Moreover, such inadequate ventilation enormously predisposes to the occurrence of post-operative collapse and chest complications (Fig. 3).

This figure is a blood pressure curve in a patient anæsthetised with curare and thiopentone. At (A) the ventilation was inadequate, although the patient was still breathing, the blood pressure rises although the patient appeared to be perfectly oxygenated. At (B) full artificial ventilation was instituted with the restoration of the normal blood pressure.

These facts are too little recognised by anæsthetists and explain many of the troubles which have been experienced when curare is used. Aided respiration—that is, increase in the tidal exchange by manually squeezing the rebreathing bag of the anæsthetic apparatus—must always be carried out when curare is used.

Acetylcholine appears to perform some function in the transmission of nerve impulses through the ganglia of the autonomic nervous system, and possible through all the synapses of the central nervous system. In the same way that curare prevents the acetylcholine-receptor substance union at the neuromuscular junction, so too does it in the autonomic nervous system.

Although this is a secondary effect, exerted perhaps only in the presence of large doses, yet it may well be of importance clinically. In the first place, curare appears to depress markedly the laryngeal and bronchial reflexes, preventing that troublesome complication and bane of the anæsthetist, spasm. Secondly, the effect on the gut has been of some interest (Gross and Cullen, 1945). I have noted marked contraction and activity of the gut in some cases but in others, including most of those anæsthetised only with a barbiturate and curare, this irritability has been absent. It is difficult to come to any conclusion in this matter, as the gut reaction will vary with the premedication and the anæsthetic agents. All the anæsthetic and narcotic drugs, not to mention atropine, act on the autonomic ganglia, and it may well be that one is observing a summation of all these effects in conjunction with curare.

Salivation has been a feature in the human subject when curare has been given without adequate premedication with atropine. This can be very troublesome for the secretion is particularly thick, glairy and tenacious. For this reason atropine should always be given in good time and in adequate dosage.

The action of curare on the central nervous system still requires further elucidation. Whittacre and Fisher (1945), in America, reported a loss of consciousness after large doses of Intocostrin and actually performed operations on three patients without any other anæsthesia. It is difficult to see how, in the completely paralysed patient, these workers knew when consciousness was lost, and in human volunteers, using even larger doses, I have been unable to produce any signs of loss of consciousness or any interference with the sensory paths in the central nervous system. Anoxia would appear to be the cause of the unconsciousness in Whittacre and Fisher's patients.

However, curare does appear to produce some " potentiation " of the anæsthetic agents. We have noticed, particularly when the barbiturates

have been used as the anæsthetic agent, that their effect is greatly enhanced by the coincident injection of curare. This applies also to other anæsthetic agents and the American workers advise that when ether is used the dose of curare must be reduced to one-third of what otherwise would be given. The potentiation of the anæsthetic agents by curare, or of the curare by the anæsthetic agents, must always be kept in mind, and it is useful in that a light plane of anæsthesia can be maintained with the minimum quantity of toxic anæsthetic agent.

Present pharmacological opinion is that the liver and kidneys are completely unaffected by curare, but its action on the heart and cardio-vascular system is a matter of some importance. The information available

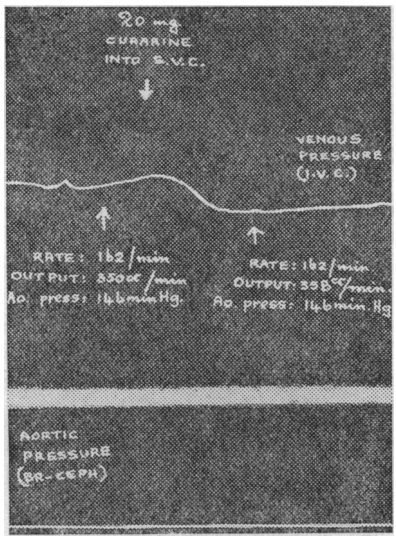

Fig. 4

Kymograph tracing of the venous pressure in a Starling heart-lung preparation (dog). Note the fall in venous pressure after the addition of d-tubocurarine chloride.

up to now has been of doubtful value, for one has never been quite certain which particular extract or preparation was being used. Certain extracts of curare, curine in particular, are known to be potent cardiac poisons. However, our clinical results led us to believe that the drug might have some effect on the myocardium. I have observed the effect of tubocurarine on the electrocardiogram in a number of cases in the human subject, and have estimated the result when this material is injected into the Starling heart-lung preparation in dogs. We can say that d-tubocurarine chloride produces no alteration in the electrocardiogram in

doses in which it is used clinically, and on injection into the heart-lung preparation in dogs, using a dosage vastly greater than anything ever likely to be used in humans, no effect whatever has been observed on the cardiac rate, output, or on the venous pressure, nor was there any interference with the coronary flow. The venous pressure is in fact consistently lowered in this preparation when curare is added (Fig. 4) (Gregory and Gray, 1947).

It must be emphasised that, in spite of this, curare must be used only with the greatest possible care to maintain full and very complete oxygenation, otherwise the patient's condition will rapidly deteriorate. Especially is this so in those cases having a poor myocardium, for they cannot cope with any sub-oxygenation. In clinical dosage, little or no effect can be observed on the blood pressure. Depression may be seen when a large dose of curare is injected quickly, especially if in association with a barbiturate (Fig. 4).

Rationale for the use of Curare in Anæsthesia

Before examining in detail the techniques which we have found to be most efficacious in the administration of curare it is first necessary to discuss the rationale underlying the use of this substance in anæsthesia, and the range of dosage which will be required.

Curare is used in anæsthesia for four purposes :—

1. To provide, using only very light anæsthesia, the muscular relaxation which is required for abdominal surgery ;
2. To facilitate, in a light plane of anæsthesia, control of the respiration during thoracic operations ;
3. To ensure freedom from laryngeal spasm during any anæsthesia ;
4. To potentiate the anæsthetic agents so that light anæsthesia can be maintained with only minimal quantities.

This rationale calls for an entirely new conception of general anæsthesia. Many of our old ideas must give way to new. The age of deep anæsthesia with all its attendant evils has passed. The administration of heavy doses of the toxic anæsthetic agents to produce satisfactory operating conditions for the surgeon resulted in post-operative depression, shock, toxæmia often manifested by prolonged nausea and vomiting, and a high incidence of pulmonary complications. Now, when curare is used, the patients are given only minimal doses of the anæsthetic agents, just sufficient to keep them asleep. They are often awake at the close of the operation, are co-operative and fully conscious on return to the wards, they are able to cough effectively and breathe freely, facts which must reduce greatly the incidence of post-operative complications. They are for the most part singularly free from those unpleasant toxic sequelæ which have always resulted from the administration of a general anæsthetic.

The new conception of light anæsthesia and adequate curarisation to produce good operating conditions must always be borne in mind, for there is no more harmful combination than curare and deep anæsthesia.

Dosage and Technique of Administration

The dosage which is employed varies from individual to individual. The aged require greatly reduced doses as also do children, and whilst a dose for weight scale is useless in adults, in children it is of great value, for in them the muscle mass upon which depends the requisite dose of curare bears a more certain relationship to body weight. We have found that for children 2 mg. per stone body weight is a safe dose for induction. In healthy fit adults 15 mg. is used for induction, but in patients over 65-70 years of age 10 mg. is safer. Owing to the possible danger of idiosyncrasy and the variability in the individual reactions of patients, one-third of the induction dose, *i.e.*, 5 mg. in healthy adults, is first given intravenously, followed by a pause of three minutes before administering the remaining 10 mg. In this way some assessment of the individual's reaction to the drug can be made. The usual reaction to this small trial dose is mainly subjective. The patients feel " drowsy," " heavy " or weak. There is a little weakness of the eye muscles but usually no definite ptosis or diplopia, and certainly no embarrassment of the respiratory function. Any reaction in excess of this is an indication either for abandoning its use in that particular patient or for modifying considerably the dosage, depending on the degree of over-reaction. Such exaggerated reactions will be seen in elderly patients, in those with a tendency to myasthenia gravis, and in the presence of true idiosyncrasy, which is rare, only one case having so far been reported.

It will be noted that the curare in our technique is given first before the induction of anæsthesia. This is done for two reasons. The first is to allow the effect of a trial dose to be assessed, and the second is to assure that the effect of the curare is present simultaneously with the induction of anæsthesia. Laryngeal spasm is thus avoided and an airway or endotracheal tube can be inserted at once. As the induction of anæsthesia is always with an intravenous barbiturate and follows immediately on the administration of the full induction dose of curare the patient experiences no discomfort.

After the initial dose small increments of d-tubocurarine are given as required. There is a cumulative effect, so that the subsequent doses are much smaller than the initial dose.

Three main techniques are employed. The first is used for the induction of anæsthesia, for endoscopies such as bronchoscopy, œsophagoscopy or laryngoscopy prior to endotracheal intubation. This technique is also useful for cystoscopy or sigmoidoscopy in patients who are resistant to ordinary doses of the intravenous barbiturates.

After the intravenous injection of the dose of curare, 2-3 ml. of sterile saline are injected followed immediately by 0·5 g. of thiopentone. After this injection the respiration becomes very shallow or may cease altogether, the jaw is completely relaxed. Respiration is usually resumed before any degree of cyanosis or harmful anoxia can occur. The pulse rate, respiration and colour of the patient must always be under observation and oxygen must be administered. This latter is most easily done through the side tube of a bronchoscope, through a catheter introduced into the trachea in the case of œsophagoscopies and in other cases through a pharyngeal airway. At any time any cyanosis must be countered by the immediate institution of artificial respiration.

With this technique patients usually recover their protective reflexes and have full respiratory function in seven to ten minutes : but should this not occur they are retained in the theatre until there is no longer any fear of respiratory depression or obstruction. If necessary prostigmine is given. For prostigmine to be effective it must be given in adequate dosage. 3-5 mg. is the usual dose given together with atropine gr. 1/50 in order to overcome the undesirable parasympathomimetic effects. With this technique all the reflexes are present at the end of the examination which is a feature of great value in bronchoscopy, especially if a biopsy has been taken.

For longer procedures after the induction of anæsthesia the patient is maintained in a light plane of anæsthesia using one of the intravenous barbiturates, usually thiopentone. The Tubarine produces the necessary relaxation or control of the respiration and at the same time by its " potentiation " effect reduces the amount of the barbiturate which has to be used so that there is no delay in recovery. After the induction has been carried out in the way which has been described, an airway or endotracheal tube is introduced and the face-piece of a closed circuit anæsthetic machine fixed in position. Further doses of curare or barbiturate are added as required. It is usually necessary to give 5-10 mg. before the peritoneum is opened or, in the case of thoracic operations, just prior to the opening of the chest. It is desirable to deepen the anæsthesia slightly when there is excessive manipulation or any stimulation of the deep abdominal or mediastinal reflexes. This is best achieved by the

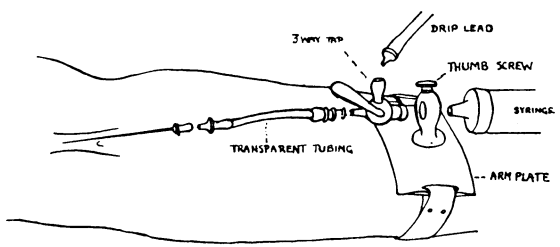

Fig. 5
Three-way tap and arm support (Halton)

addition of a little cyclopropane to the circuit. As soon as the stimulation ceases the anæsthesia can be lightened quickly, so that there is no undue depression of the patient or delay in recovery.

As solutions of tubocurarine and the barbiturates are incompatible and a precipitate is formed when they are mixed it has been necessary to devise special apparatus for their administration. Two types of apparatus are used. The first is simple (Fig. 5), and consists merely of an arm-plate, three-way tap, and a piece of transparent tubing. To use this an arm must be available and as this is not always possible a more elaborate apparatus has been devised (Fig. 6). This consists of a special combina-

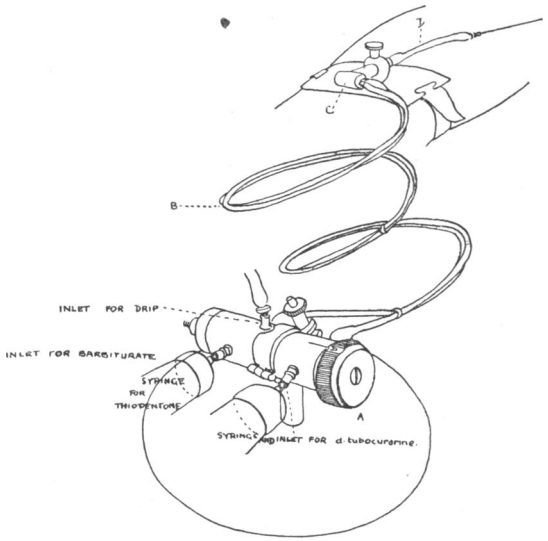

Fig. 6
Remote control tap (Gray, Osborne)

tion tap with inlets for an intravenous continuous drip, the curare and the barbiturate. The barbiturate passes down one tube and the drip through the other. The tubocurarine is put into the drip side of the apparatus and mixing is thus avoided. Owing to the firm fixation of the needle by the arm-plate the arm can be placed in any position, usually by the side of the patient.

If it is not desired to give continuous barbiturate anæsthesia the procedure is the same but one uses only the inhalational agents.

Some writers have suggested the use of a preliminary induction dose of tubocurarine with the maintenance of curarisation by a subsequent intramuscular injection. The effects of such an intramuscular injection come on after an uncertain interval and last for an indefinite time. As the aim is to produce curarisation only when it is required the intra-

muscular route would appear unsatisfactory on *a priori* grounds, and one which must give rise to trouble.

If you will allow me, I must repeat once again that control of the respiratory exchange is essential when this substance is used in order to ensure that oxygenation is adequate and carbon dioxide eliminated. This control is best attained by use of a closed circuit. The circuit must be completely free from leaks and the application of the anæsthetic mask to the face made really air-tight.

As has been pointed out, when tubocurarine or Intocostrin is used in effective dosage the tidal exchange is reduced and must be restored by rhythmic manual compression of the rebreathing bag of the anæsthetic machine. Adequate ventilation of the lungs is always possible through a pharyngeal airway of good design. Endotracheal intubation is, however, probably advisable as a routine when beginning to use this technique and it must always be employed in the following cases.

First, when there is any gastric or intestinal obstruction or when such a condition might be suspected. One of the dangers is an insidious or sudden regurgitation of stomach or intestinal contents with their subsequent aspiration into the bronchial tree. The œsophageal muscles and sphincters are relaxed by the tubocurarine and thus such an accident is facilitated. This has proved a real danger against which one must always be on guard.

Secondly, in thoracic operations where bronchial occlusion or suction drainage is likely to be required.

Thirdly, where the site or nature of the operation precludes the use of an anæsthetic mask.

Fourthly, where there is tracheal or laryngeal obstruction, and finally, when an overdose has been administered or there are other difficulties.

Two problems remain to be considered. The ordinary signs of anæsthesia are modified by the use of tubocurarine. The eye reflexes are sluggish and the respiratory signs are invalidated but three indications of a lightening anæsthesia remain. There is no danger of a patient becoming conscious during the operation and being unable to move because of the paralysis. In the doses which are recommended complete paralysis will not ensue and a lightening anæsthesia will be indicated by slight movement of a limb or a tendency to phonation. There may also be an increase in the respiratory rate and depth. If respiration has been completely abolished a rising pulse rate will indicate an inadequate anæsthesia. The need for the administration of more curare will be indicated by unsatisfactory operating conditions, by an increasing depth of respiration and by the degree of resistance to pulmonary inflation by pressure on the rebreathing bag.

One tries at the end of the operation to have a conscious patient and one with full and adequate respiratory function. For this reason it is preferable to obtain relaxation for the final closure of the abdomen by deepening the anæsthesia slightly with cyclopropane rather than by the

administration of more curare. Very little deepening is required as the effect of the curare which has already been given is enhanced by the further administration of an anæsthetic agent.

A marked feature has been the freedom from shock in these patients, and this despite the lightness of the anæsthesia, provided that it is deepened a little when there is gross stimulation of the deep reflexes. It has been suggested that curare gives some protection against shock. I do not believe this, but am sure that the fact that the patient's vital centres are less depressed when light anæsthesia is used and that as a result these, particularly the vasomotor centre, are better able to cope with any circulatory changes which may occur.

Tubocurarine is, I believe, a notable advance in our specialty, and one to which already very many patients are indebted for their lives, but it must never be forgotten that this is a very potent and dangerous drug and one having a profound and significant effect on the respiratory function. While the only two absolute contra-indications to its use appear to be the presence of myasthenia gravis or organic respiratory obstruction, it should never be used by those who are not used to dealing with the apnœic patient. The results which can be obtained fully justify the time spent in studying and gaining the special experience which is necessary.

REFERENCES

BERNARD, C. (1857) Leçons sur les effets des substances toxiques.

BOEHM, R. (1895) *Arch. f. exper. Path. u. Pharmak.* **35,** 16.

BOEHM, R. (1897) *Arch. Pharmak.* **235,** 660.

BROWN, G. L., DALE, H. H. & FELDBERG, W. (1936) *J. Physiol.* **87,** 394 ; 424.

COUNCIL OF PHARMACY AND CHEMISTRY (1945) *J. Amer. Med. Assoc.* **129,** 517.

D'ACUGNA, C. (1642) Nuevo descrubimiento de Gran Rio de las Amazonas.

DALE, H. H., FELDBERG, W. AND VOGT, M. (1936) *J. Physiol.* **86,** 353.

ECCLES, J. C., KATZ, B. & KUFFLER, S. W. (1941) *J. Neurophysiol.* **4,** 362.

GILL, R. C. (1946) *Anesthesiology,* **7,** 14.

GREGORY, R. A. AND GRAY, T. C. (1947) In the press.

GROSS, E. G. AND CULLEN, S. C. (1945) *Anesthesiology,* **6,** 231.

HUMBOLD, A. VON AND BONPLAND, A. (1821) Personal narrative of travels to the Equinoctial Regions of America during the years 1799-1804.

KING, H. (1935) *J. Chem. Soc.,* Pt. 2, 1381.

RALEIGH, SIR W. (1595) Discovery of Guiana. (Included in Hakluyt's Principal Voyages and Discoveries of the English Nation, 1599-1600.)

SCHOMBURGK, Sir R. H. (1857) *Ann. Nat. Hist.* **7,** 423.

WATERTON, C. (1812) Wanderings in South America.

WHITTACRE, E. J. AND FISHER, A. J. (1945) *Anesthesiology,* **6,** 124.

WINTERSTEINER, O. AND DUTCHER, J. D. (1943) *Science,* **97,** 467.